INTRACELLULAR POTENTIALS OF CULTURED RECTAL GLAND CELLS OF Squalus acanthus

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Electrolyte transport of cells in culture has been examined in several established lines of absorptive epithelia (LLC-PK_1,MDCK,A6), but studies of the properties of secretory epithelia in culture are much more limited. We have previously shown that dispersed tubules of the rectal gland form monolayers in culture that are viable 4-5 days (Farmer and Forrest, MDIBL 23:1983). A special advantage of the rectal gland cell in culture is its large size, up to 60 um, which facilitates the placement of intracellular electrodes. In the present abstract we further examine the conditions for primary growth and report intracellular potentials under several conditions.

Rectal glands were aseptically removed from Squalus acanthus, minced and washed in shark Ringer's with antibiotics. Fragments were digested in collagenase and hyaluronidase (764 units/ml,1200 units/ml) up to 4 hours, and trypsin (0.05% to .25%) up to 2 hours at 4°C. Minced tubules and dispersed cells were plated on tissue culture plastic and collagen coated surfaces in medium CL2. Levels of NaCl, KCl, glutamine, urea, trimethylamine oxide and fetal calf serum were experimentally varied. Growth was measured by the expansion of monolayer size with time and viability was indexed by trypan blue exclusion. Cells grown on both collagen and plastic were impaled from the apical surface using standard micropuncture techniques.

Two factors were shown necessary for the initial formation of monolayers from tubule fragments: trypsinization and the presence of calcium in wash and digest Ringer's. Table 1 outlines the percentage of viable monolayers which developed with different treatments. In 11 preparations of cells on semisolid collagen, hollow spheres or lumina developed similar to those described in MDCK cells (Hall,G. et al.,Proc.Nat.Acad.Sci.79:4676.1982). Intracellular potentials are shown in Table 2. Cells pretreated with dibutyryl cAMP (10^{-4}M)+ 2-chloro-adenosine (10^{-4}M) were hyperpolarized by the addition of furosemide (10^{-4}M)(-3.75$\pm$ 1.16 mV, n=8).

Intracellular potentials of cultured cells are lower than those measured in isolated tubules (-50 vs.-80mV), but furosemide induced hyperpolarization of stimulated cells is comparable (-3.75 vs. - 4mV). These results indicate the presence and activity of furosemide sensitive Na/K/Cl carriers, and support the feasibility of the use of cultured cells in electrophysiological studies.

TABLE 1

GROWTH RESULTS

(% of individual plates of a given treatment with viable monolayers)
(n = total number of plates)

Urea +TMAO	
250 mM Urea +50 mM TMAO	79% (29)
350 mM Urea +100 mM TMAO	18% (11)
pH	
Open, unbuffered flasks	33% (15)
Closed flasks + HEPES	83% (15)
1% CO_2, no HEPES	80% (10)
Temperature	
20 to 26°C	70%(10)
16°C constant	20%(10)
20°C constant	78%(18)
% Fetal Calf Serum	
2%	73%(15)
10%	52%(23)

TABLE 2

INTRACELLULAR POTENTIALS OF RESTING CELLS
(mV ± S.E.) (n)

Surface	24 Hours	48 Hours	72-96 Hours
Plastic	-40.0 ± 4.5(9)	-32.6 ± 1.2(5)	-32.8 ± 3.5(13)
Collagen	-50.1 ± 1.8(12)	-48.1 ± 3.5(16)	-47.0 ± 2.0(10)